

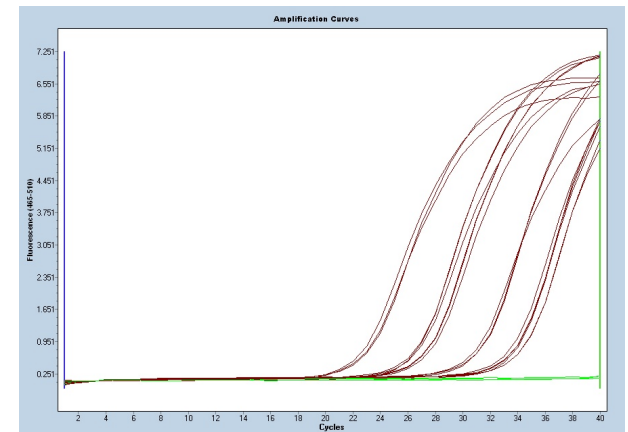
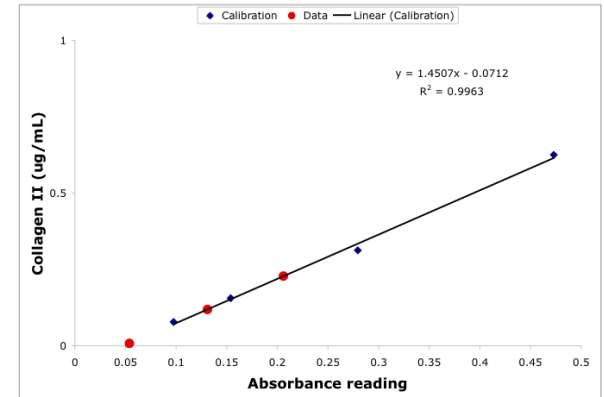
Cartilage TE: from *in vitro* and *in vivo* models to the clinic

Module 3, Lecture 6

20.109 Spring 2011

Lecture 5 review

- What are some advantages of ELISA as a protein assay?
- Compare qPCR and end-point RT-PCR as gene expression assays.

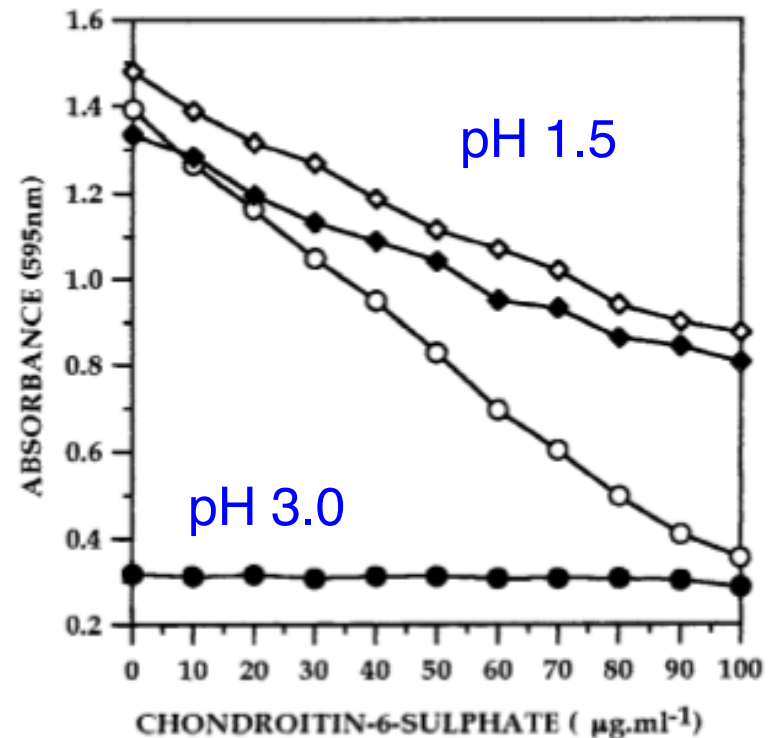


Topics for Lecture 6

- Proteoglycan assay
- qPCR analysis
- Cartilage TE *in vitro*
- Cartilage TE *in vivo*
- Cartilage TE in the clinic

Proteoglycan content assay

- DMMB cationic dye binds (-) groups on PGs
- GAG sulfates detected at pH 1.5-3.0
- Alginate carboxyls detected at pH 2.0-3.0
- Complex causes short-lived A_{595} peak reduction
- Figure shows PG standard in presence of alginate

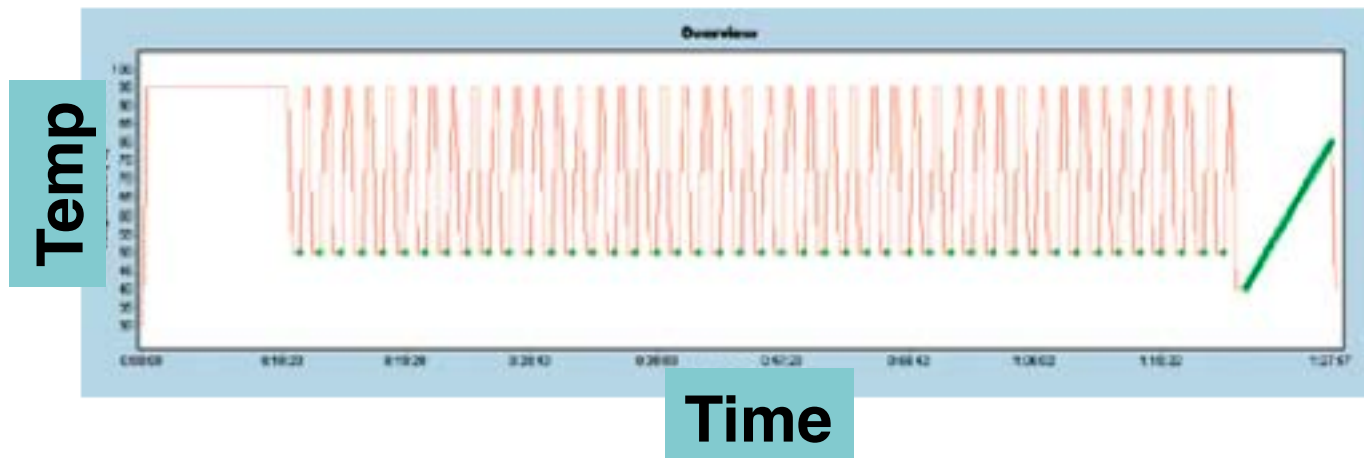


Enobakhare, et al., *Anal Biochem* **243**:189 (1996)

qPCR cycling

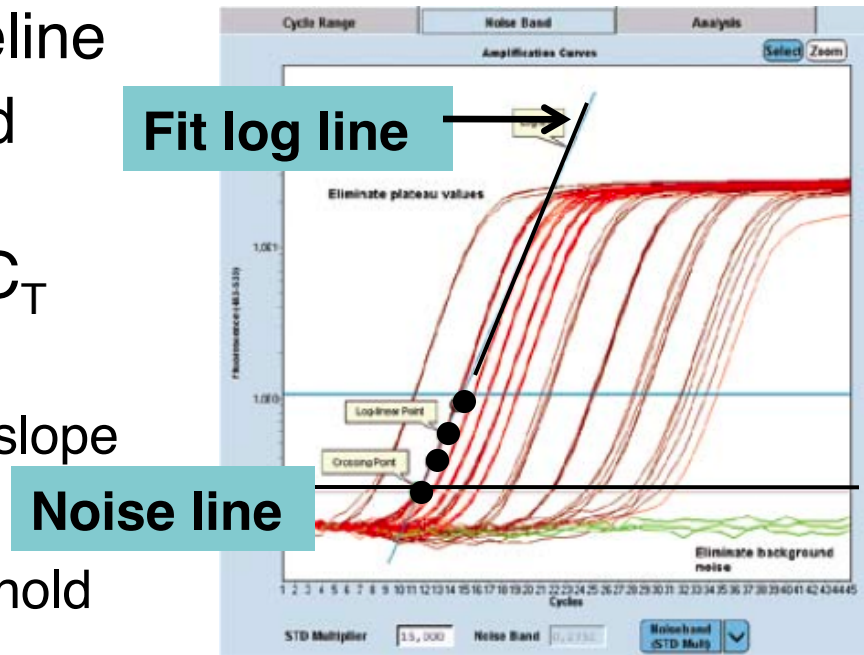
- Melt DNA, activate hot start enzyme, 10 min at 95 °C
- 40 PCR cycles: melt (15 sec at 95 °C); anneal/extend
- Anneal/extend ≤ 1 min at 60 °C
 - 2-step cycling often sufficient (short products)
 - take single fluorescence snapshot at end
- Melting curve
 - slowly heat to 95 from 60 °C
 - continuously measure fluorescence

Image from Roche manual



qPCR threshold cycle C_T

- Initial cycles used to set baseline
- C_T = intensity \gg background
- Two main ways to calculate C_T
- 2nd derivative maximum
 - each C_T identified by largest Δ slope
- Fit points
 - all C_T s identified by same threshold
 - linear regression in log phase
 - recommended for our analysis type

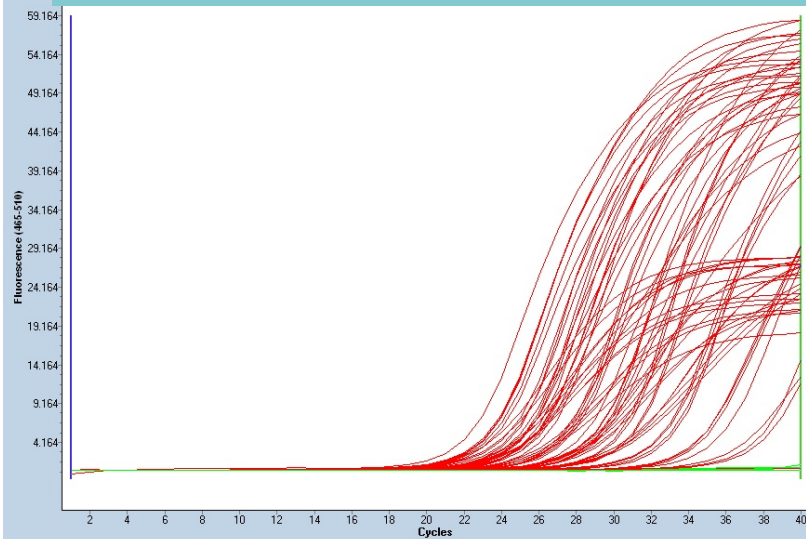


From Roche manual

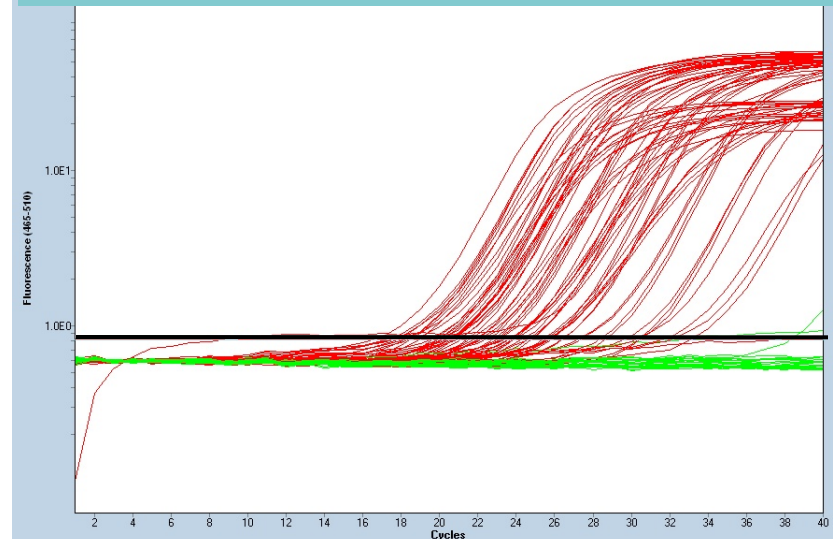
Roche, LightCycler 480 Operator's Manual, software version 1.5

T/R qPCR data

Raw fluorescence vs cycle #



Log scale with noise threshold



qPCR relative expression analysis

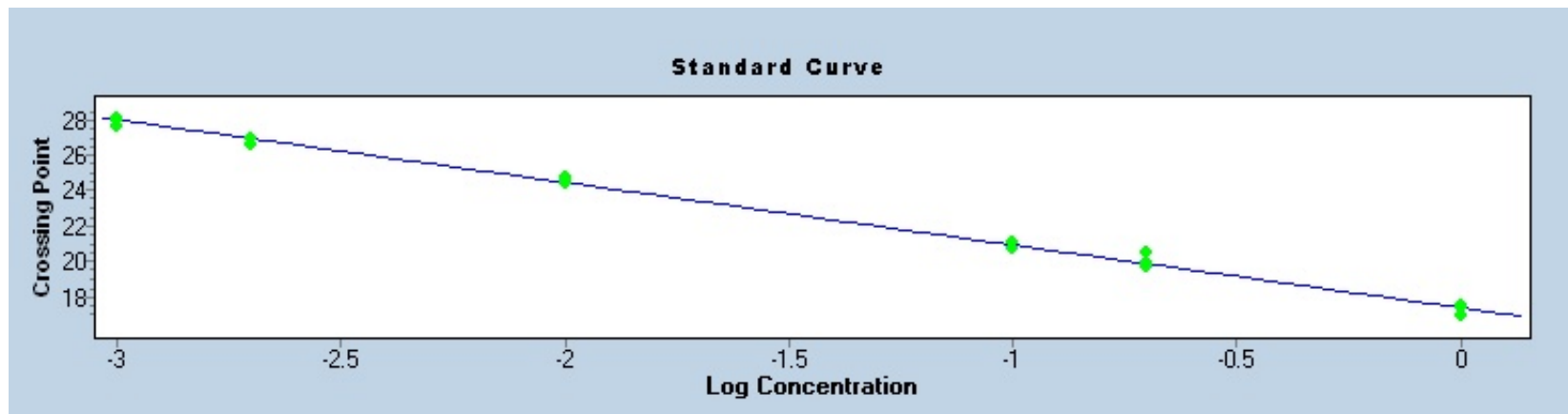
- Relative gene expression analysis
 - Control for cDNA amount with reference (e.g., 18S rRNA)
 - Expression change relative to a control (e.g., fresh cells)
- E is amplification efficiency for that primer set

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta CP_{\text{target}}(\text{control} - \text{sample})}}{(E_{\text{ref}})^{\Delta CP_{\text{ref}}(\text{control} - \text{sample})}}$$

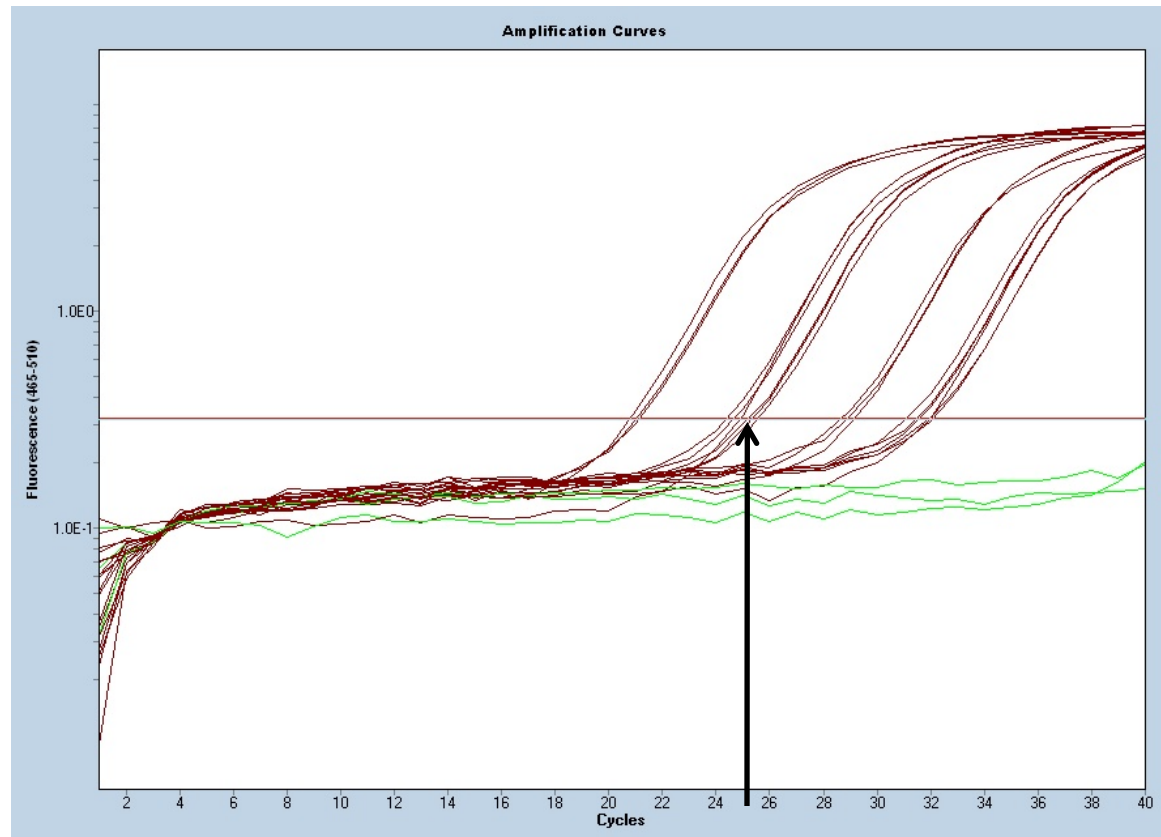
Equation 1 from M.W. Pfaffl, *Nucleic Acids Res* **29**:2002 (2001)

qPCR primer set standard curves

- Slope indicates primer amplification efficiency
 - $E = 10^{(-1/\text{slope})}$
 - $E = 2$ for slope = -3.3
- Measure samples over 3-5 logs, in triplicate



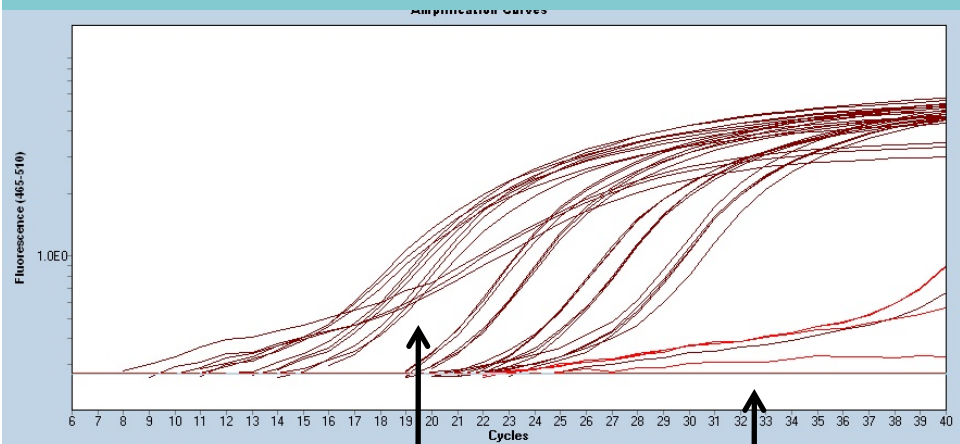
Detection limit for change



2-fold change detectable but C_T error/scatter may overlap

Optimizing primer concentration

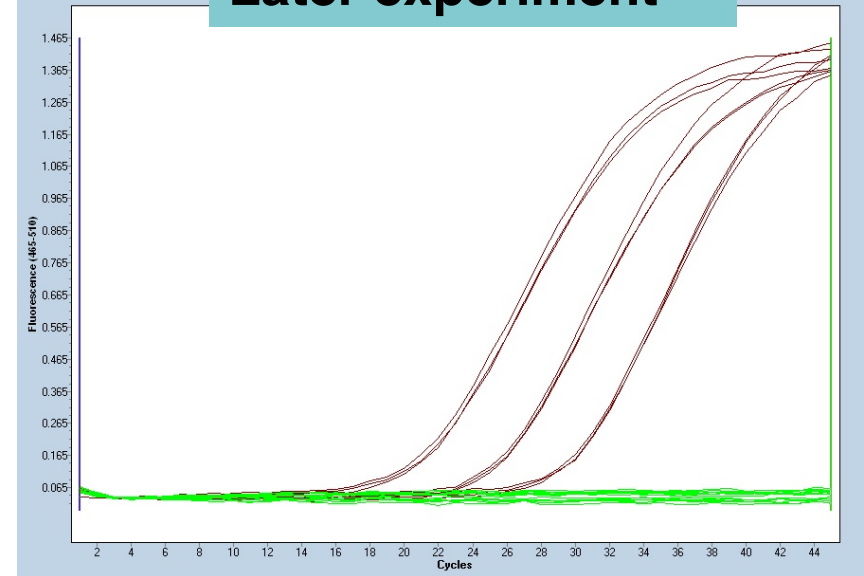
First experiment – too high [primer]



High [cDNA] sample
oddly shaped

No-template controls give
primer-dimer product

Later experiment



Great replicate agreement
and flat controls (green)

Interlude

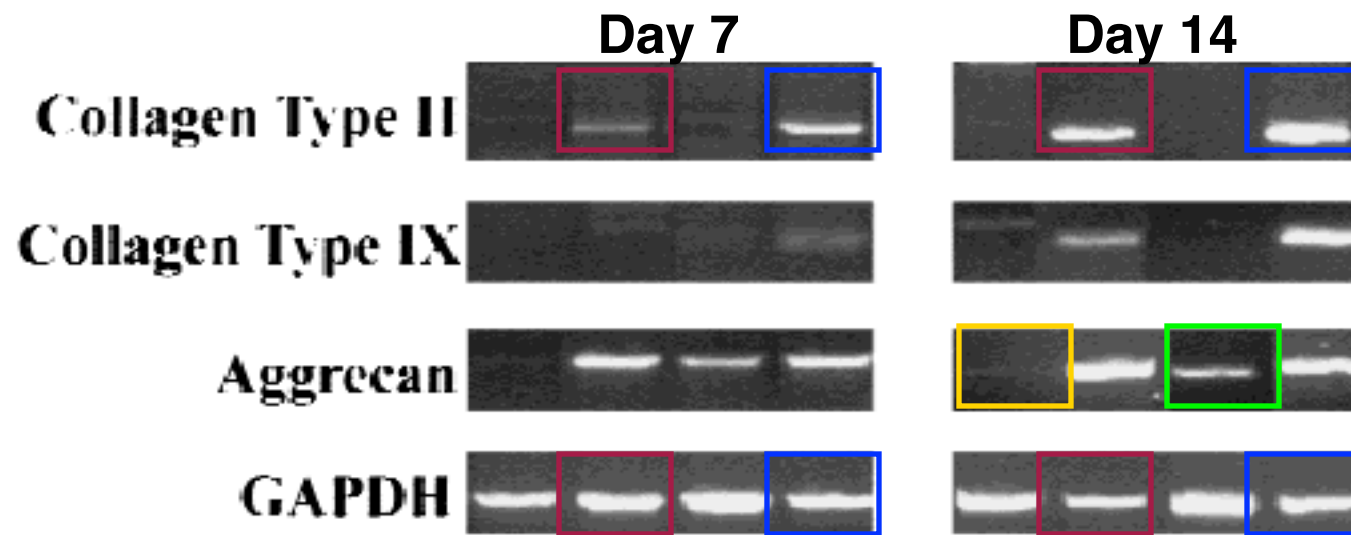
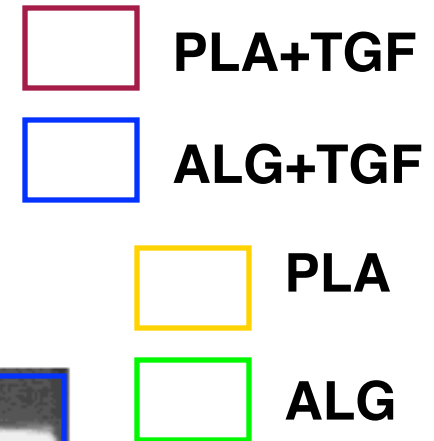
Lecture 8: your choice of TE topics (list on board)

Which one is cuter? Tree kangaroo or human baby?

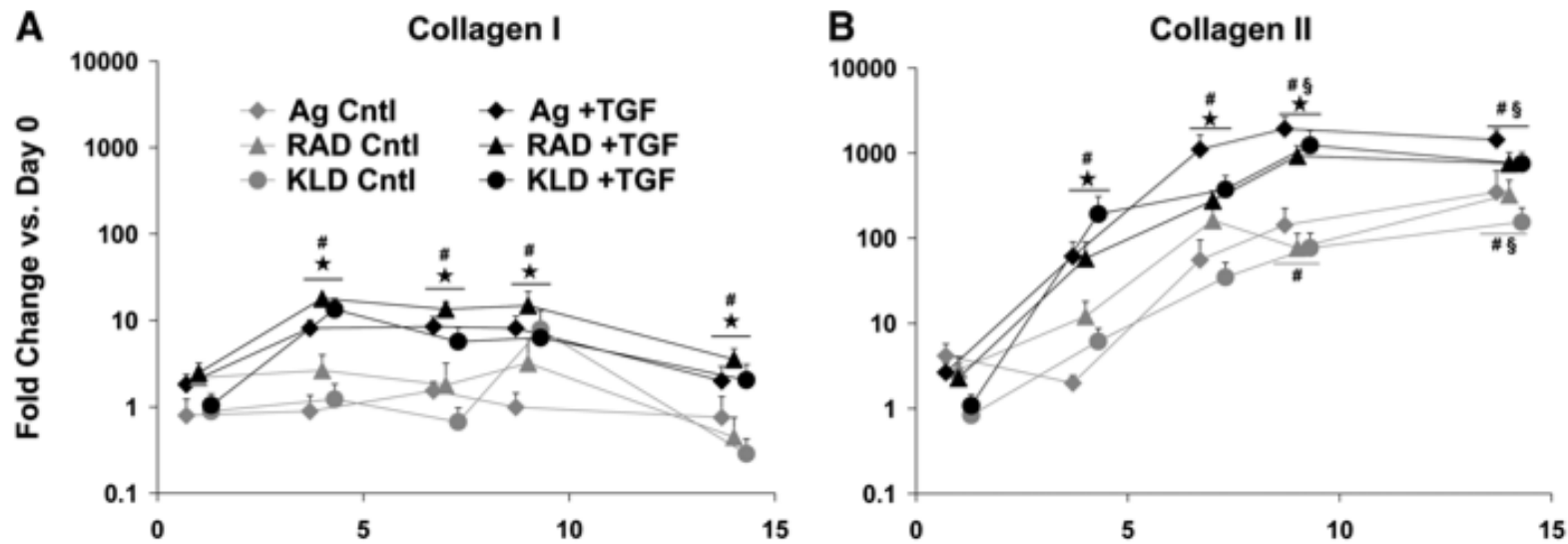


Chondrogenesis *in vitro*

- Porous PLA scaffold w/ or w/out alginate
- Alginate alone somewhat chondrogenic
- Alginate+TGF better than PLA+TGF



Recent Grodzinsky lab work shows merits of peptide gels



CN II expression of stem cells increases in agarose, RAD, and KLD gels over 5-10 days

Kopseky et al., *Tissue Eng A* **16**:465 (2010)

Scaffold-free *in vitro* cartilage TE

- Method: rotational culture of rabbit chondrocytes with no cytokines
- Results
 - Mostly dynamic culture gave best results: low apoptosis, very rigid disc
 - Fresh ECM made: primarily CN II and PG
 - Organized architecture, similar to *in vivo*
- A scaffold-free method is inherently biocompatible
 - Any disadvantages?
 - Pros/cons of *cell-free* methods?

T. Nagai et al., *Tissue Eng* **14** (2008)

Static



Dynamic, 3 d

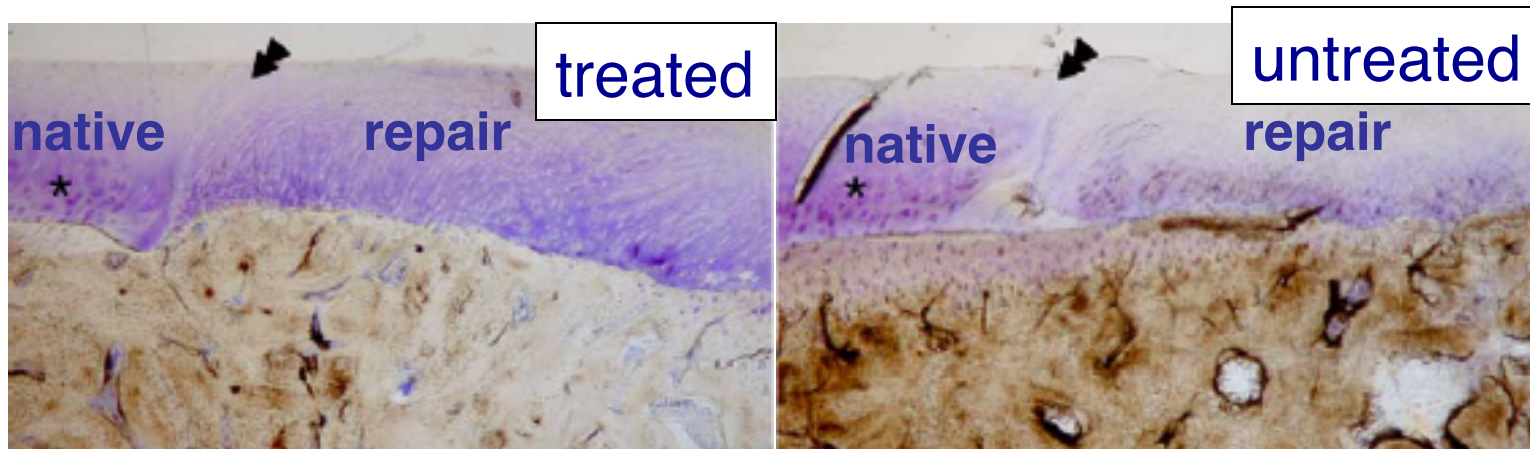


Dynamic, 3 w



Large animal *in vivo* model

- D. Barnewitz et al. *Biomaterials* **27**:2882 (2006)
- Biodegradable scaffold with autologous cells
- Examined horses and dissected joints after 6-12 months
- Matrix synthesis, implant integration with native tissue
- Why use a large animal model (vs. small)?



Advantages of working *in vivo*

- Ability to mimic human disease-state
- Ability to mimic therapy/surgery applied to humans
 - especially true for large animal models
- Can compare results to “gold standard” treatment
- The construct interfaces with an actual wound, the immune system, etc. - more realistic environment
- Toxicity studies more meaningful

Cartilage pathology

- Cartilage has little regeneration capacity – why?
- Early damage can promote later disease
- Osteoarthritis pathology
 - PG and collagen loss, PG size ↓
 - ↑ water content, ↓ strength
 - chondrocyte death
- Symptoms
 - loss of mobility
 - pain



Image from OPML at MIT: <http://web.mit.edu/cortiz/www/AFMGallery/AFMGallery.html>.

V.C. Mow, A. Ratcliffe, and S.LY. Woo, eds. *Biomechanics of Diarthrodial Joints* (Vol. I) Springer-Verlag New York Inc. 1990

Treatments for cartilage damage

- **Strategy 1: enhance/provoke healing**
 - biologics: hyaluronic acid, TGF- β , etc.
 - damage bone (stem cell effect)
- **Strategy 2: replace tissue**
 - joint replacement
 - synthetic or donated tissue
 - invasive or fiber-optic (partial)
 - cell and/or scaffold implantation
 - immature therapy
- **Other/supplemental**
 - mechanical, electrical stimulation
 - debridement (rid debris)



Public domain image
(Wikimedia commons)

S.W. O'Driscoll. *J Bone Joint Surg* **80**:1795 (1998)

S. Poitras, et al. *Arth Res Ther* **9**:R126 (2007)

C.M. Revell & K. A. Athanasiou. *Tissue Eng Pt B-Rev* **15**:1 (2009)

Cutting edge of treatment

- Cell-based therapies on the market (e.g., Carticel)
- Scaffold-based approaches in trials (e.g., NeoCart, INSTRUCT)

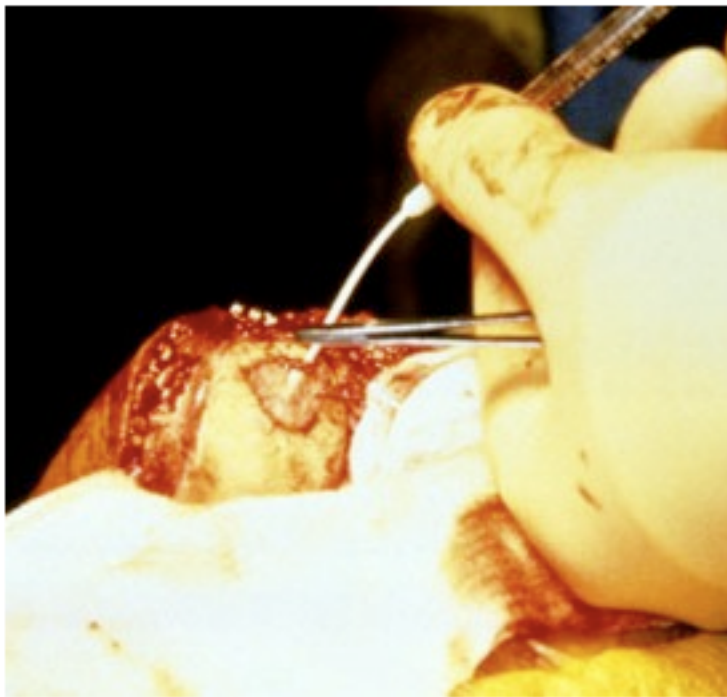


Figure 21: Injecting Carticel under periosteal patch

2. Tissue Production
Cells grow on a patented 3D matrix in a tissue engineering processor under conditions that simulate those in the body. >



3. NeoCart Implant
NeoCart has the characteristics of native articular cartilage. <

Many clinical trials are ongoing

Found 186 studies with search of: cartilage

[Hide studies that are not seeking new volunteers.](#)

[Hide studies with unknown recruitment status.](#)

Rank	Status	Study	
1	Recruiting	Cartilage Autograft Implantation System (CAIS) for the Repair of Knee Cartilage Thro Conditions: Articular Cartilage Injury; Osteochondritis Dissecans Interventions: Procedure: Microfracture; Procedure: Cartilage Autograft Im	← scaffold + own tissue
2	Active, not recruiting	Evaluation of an Acellular Osteochondral Graft for Cartilage LEsions Pilot Trial Condition: Articular Cartilage Injury Interventions: Device: Kensey Nash Corp. Cartilage Repair Device; F	← scaffold alone
3	Recruiting	AS902330 in Cartilage Injury Repair (CIR) Condition: Isolated Cartilage Injury of the Knee Interventions: Drug: AS902330; Other: Placebo	← drug alone
4	Completed	The Objectives of the Cartilage Repair Registry is to Report Long Term Efficacy and Safety of Cartilage Repair Pr Conditions: Articular Cartilage; Cartilage Diseases Intervention: Biological: Carticel (autologous cultured chondrocyte) in	
5	Recruiting	Study to Compare the Efficacy and Safety of Cartistem® and Microfracture in P Conditions: Cartilage Injury; Osteoarthritis Interventions: Biological: Cartistem; Procedure: Microfracture treatm	← cord blood stem cells in gel
6	Not yet recruiting	Follow-Up Trial Comparing BST-CarGel and Microfracture in Repair of Articular Cartilage Lesions in the Knee Condition: Articular Cartilage Lesions in the Knee	

Screenshot from www.clinicaltrials.gov, May 2011

Lecture 6: conclusions

- Both *in vitro* and *in vivo* models of cartilage repair can reveal valuable insights, but have different strengths.
- Cell-based therapies have come to market for cartilage TE, and scaffold-based therapies are on the horizon.

Next time: Atissa on presenting with a partner; research proposal open discussion.

Lecture 8: special topics in TE.